

Application No. 10/658,111  
Response dated March 18, 2005  
Reply to Office Action of September 30, 2004

# Exhibit 6

**INHIBITION OF P38 MITOGEN-ACTIVATED PROTEIN KINASE SUPPRESSES  
INTERLEUKIN-1 $\beta$ -EXPRESSION AND PREVENTS PROGRESSION OF  
CARDIAC HYPERTROPHY AND CONGESTIVE HEART FAILURE IN RATS.**

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**Background:** Proinflammatory cytokines have been reported to participate in cardiac hypertrophy and congestive heart failure (CHF) induced by mechanical overload. Intercellular signaling leading to proinflammatory cytokine production involves p38 mitogen-activated protein kinase (MAPK). We evaluated the effects of a p38 MAPK inhibitor in Dahl salt-sensitive rats (DS) with hypertension-induced left ventricular (LV) hypertrophy at risk of progression to CHF. **Methods:** DS rats were divided into four groups: a 7W in which a p38 MAPK inhibitor (FR167653; 2 mg/kg/day) was administered from age 7 weeks when we initiated a high-salt diet in all groups; an 11W in which FR167653 was administered beginning at 11 weeks, when concentric LV hypertrophy appeared; a 15W in which FR167653 was administered beginning at 15 weeks, when CHF developed; and an untreated group (UN). Dahl salt-resistant rats (DR), a control group, were not given FR167653. **Results:** At 19 weeks of age, interleukin-1 $\beta$ (IL-1 $\beta$ ) mRNA expression was significantly less in all treated groups than in UN ( $p<.01$ ), indeed nearly the same as in DR. Expression of monocyte chemoattractant protein-1 (MCP-1) mRNA and the number of macrophages in LV did not decrease in any treated group. Echocardiographical findings indicated the presence of LV concentric hypertrophy in 11W and LV wall dilation in 15W, but no such changes in 7W, and preserved LV wall motion in all treated groups. Hypertrophy of myocytes and increased interstitial fibrosis were observed in LV sections from 11W, 15W, and UN but not in 7W or DR. All UN had died of pulmonary congestion due to LV dysfunction by 22 weeks. The survival rate at 22 weeks was 90% in 7W, 50% in 11W, and 30% in 15W groups. Kaplan-Meier survival analysis demonstrated a significant improvement in 7W ( $p=.0015$ ) and 11W ( $p=.0320$ ) compared with UN. **Conclusion:** The p38 MAPK inhibitor suppressed IL-1 $\beta$  production by macrophages infiltrating the LV in response to MCP-1. The inhibitor prevented progression of cardiac hypertrophy and CHF.